hundred different targets. By advancing coarse-grained computer simulations and experimental techniques, novel mechanistic insights were gained in defining the pathways leading to recognition and for how target selectivity can be achieved at the molecular level. A model requiring mutually induced conformational changes in both calmodulin and target proteins was necessary and broadly informs how proteins can achieve both high affinity and high specificity.

3721-Pos Board B449

Protein Folding and Aggregation - From Crowded Environments into the Cell

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Protein misfolding and aggregation cause neurodegenerative disorders like Huntington, Parkinson, Alzheimer and prion diseases. The diseases may be predominantly caused by "gain-of-function" proteotoxicity, with misfolded proteins prefibrillar and fibrillar aggregates being the toxic species. The folding and aggregation kinetics are predominantly investigated in vitro in aqueous solution. We study solvent-induced effects on protein aggregation with the focus of understanding the effects of the crowded cellular environment. We use a combination of fluorescence microscopy and temperature jump relaxation to spatio-temporally resolve these events in a single living cell. We present new insights into the in cellulo aggregation pathway of the huntingtin exon-1 protein.

3722-Pos Board B450

What Kind of Microviscosity Does a Molecule Experience During its Rotational and Translational Diffusion in Crowded Environments?

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Living cells are known to be crowded with organelles, biomembranes, and macromolecules such as proteins, DNA, RNA, and actin filaments. In such crowded environments it is reasonable to believe that cellular viscosity is heterogeneous, which is likely to influence biomolecular diffusion, protein-protein interactions, protein-substrate interaction, and protein folding. In this contribution, we investigate the difference between bulk viscosity and microviscosity in crowded environments and their effects on both rotational (ps-ns) and translational (ms-s) diffusion of rhodamine green (as a probe) using time-resolved fluorescence anisotropy (TRFA) and fluorescence correlation spectroscopy (FCS), respectively. For biomimetic crowding, Ficoll-70, BSA and ovalbumin were used as crowding agents and compared with glycerol-rich solutions as a homogeneous environment. Assuming a Stockes-Einstein model, the microviscosity was calculated using TRFA and FCS, assuming no binding, and the results are compared with the bulk viscosity, which was measured using a conventional viscometer. Our results indicate that the micro- and bulk viscosities in a homogeneous environment like glycerol-rich solutions are similar over the 1-20 cP range. In Ficoll-70, BSA and ovalbumin-crowded environments, the microviscosity differs from the corresponding bulk viscosity, depending on the nature of crowding agents (i.e., proteins versus polymers) and the concentration of crowding agents. These results are discussed in terms of both non-specific binding and heterogeneous viscosity in crowded solutions, which in return provide an apparent deviation from the Stokes-Einstein model (i.e., Brownian diffusion). Our findings provide a foundation for FCS and TRFA-based studies of diffusion and binding of biomolecules in the crowded milieu of living cells.

3723-Pos Board B451

Macromolecular Crowding Effects on the Multiscale Diffusion of Single Molecules

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Macromolecular crowding in living cells influences diffusion, intermolecular interactions, protein folding and intracellular transport. We investigated the rotational and translational diffusion of chymotrypsin inhibitor 2 (CI2) and enhanced green fluorescent protein (EGFP) in biomimetic crowded environments that are created from synthetic polymers (Ficoll-70, Ficoll-400) or proteins (bovine serum albumin and ovalbumin) and compared these with diffusion in homogeneous solvents (buffer and glycerol). Time-resolved fluorescence anisotropy (ps-ns) and fluorescence correlation spectroscopy (us-s) were used to elucidate the effects of nonspecific binding on the diffusion mechanism of size-dependent tracers. The measured rotational-to-translational diffusion coefficient ratios for CI2 and EGFP indicate that the diffusion deviates from the Stokes-Einstein model (i.e., non-Brownian), depending on the type of crowding agent and the tracer. We attribute these findings to nonspecific interactions between the tracer and crowding agents, as well as microviscosity heterogeneities in the crowded environments. Our real-time, multiscale diffusion measurements for CI2 are compared with recent NMR experiment under similar experimental conditions.

3724-Pos Board B452

Protein Stability in Living Cells

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Theories concerning the effects of macromolecular crowding assert that the biophysical properties of proteins and nucleic acids can be significantly altered in native cellular environments relative to buffer alone. Despite a growing number of studies probing equilibrium thermodynamic protein stability in cells, there remains a lack of quantitative information, especially regarding residue-level stability under non-perturbing conditions. We have measured the in-cell stability of the 56-amino acid B1 domain of protein G (GB1) at the residue level without using destabilizing solutes or thermal modification by using NMR-detected hydrogen-deuterium exchange of quenched cell lysates. Comparison to dilute solution (pH 7.6 and 37 °C) shows that residues are stabilized in *Escherichia coli* cells by as much as 1.1 ±0.1 kcal/mol (Figure 1). We have also identified the residues most important for global folding of GB1 in cells. We discuss the implications of our findings with respect to structural models gleaned from studies conducted in buffer alone.

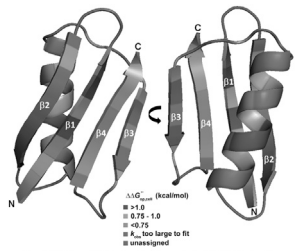

Figure 1. Residues of GB1 colored by the magnitude of stabilization in cells.

3725-Pos Board B453

AUC in Serum using the AVIV-FDS and Sedanal Global Direct Boundary Fitting

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The development of the fluorescence detection system (Aviv-FDS) for the AUC allows a single fluorescently labeled species to be quantitatively characterized against a highly concentrated and heterogeneous background. During our use of the FDS to characterize ELP, a novel drug delivery vector (see Lyons, et. al., Biophys. J. 104, 2009-2021, 2013), in serum, we encountered the Johnston-Ogston (J-O) effect. The J-O effect is a classical anomaly in sedimentation theory describing the non-ideal sedimentation properties of a component as a function of high concentrations of other components. We examined the J-O effect using recent advances in AUC hardware, the AU-FDS (AVIV Biomedical), and data-analysis methods, primarily Sedanal global direct boundary fitting. We empirically quantified the self and cross-sedimentation non-ideality properties of ELP and the two most ubiquitous serum proteins, Albumin (~35 mg/ml) and γ-Globulins (~10-15 mg/ml). We have verified and measured the presence of cross-term hydrodynamic and thermodynamic non-ideality by running SV studies on a fluorescently labeled component (~100 nM) in a titration experiment with high concentrations of unlabeled components. This has been accounted for through the introduction of a 3x3 non-ideality matrix of Ks and BM1 values into Sedanal. ELP experiments with mixtures of Albumin and γ-Globulins were also performed in an attempt to recapitulate the J-O behavior of a serum solution. Clearly other components or effects contribute to the J-O effect and additional experiments with lipids and PEG solutions are planned. These studies lay the groundwork for bringing quantitative hydrodynamic analyses into crowded environments, and will allow measurement of hydrodynamic and equilibrium macromolecular properties in a physiological state. (Supported by the UMC AUC Facility and NSF MRI grant 1040372.)

Voltage-gated K Channels III

3726-Pos Board B454

Screening of Novel Modulators for BK_{Ca} Channel by the Cell-Based Assay Platform Employing A Hyperactive Mutant Channel

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Development of a cell-based functional assay for large-conductance calcium-activated potassium (BK_{Ca}) channels has been challenging due to its unique